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(54) PRODUIT LAITIER FERMENTÉ  
(54) FERMENTED MILK PRODUCT

(57)

The invention concerns a method for making a fermented milk composition which consists in: pasteurising the milk at a temperature and for a duration such that its redox potential at 25 °C is brought down to a value less than 450 mvolt, adding agents for stabilising the milk redox, inoculating the milk with lactic bacteria, fermenting the milk until at least 106 cfu/ml and an Aw higher than 0.97 is obtained. The invention also concerns the use of milk having a redox potential at 25 °C less than 450 mvolt and containing agents stabilising this milk redox potential, for preparing a milk product fermented with lactic bacteria. The invention further concerns milk compositions packaged in a material permeable or semipermeable to oxygen, said compositions having a redox potential less than 450 mvolt and an Aw higher than 0.97, and at least 106 cfu/ml of probiotic lactic bacteria.

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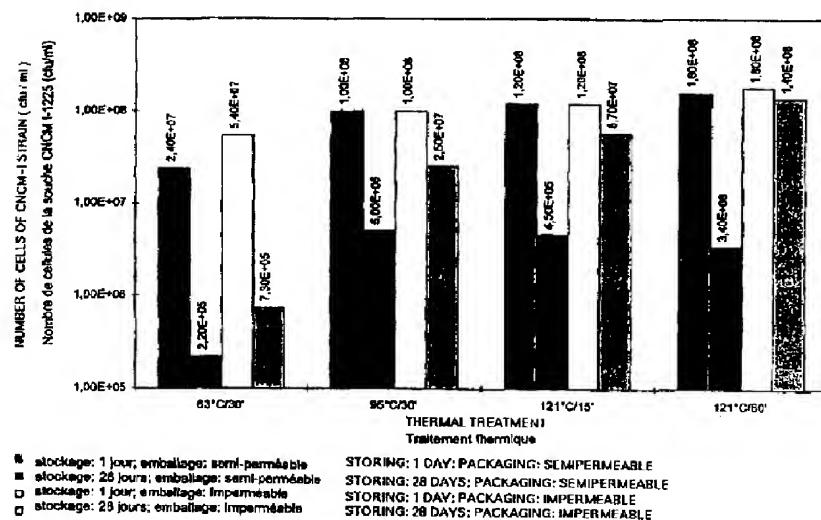
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(54) **PRODUIT LAITIER FERMENTÉ**

(54) **FERMENTED MILK PRODUCT**



(57) Procédé de fabrication d'une composition laitière fermentée dans lequel, on pasteurise un lait à une température et pendant un temps tels que son potentiel rédox à 25 °C est abaissé à une valeur inférieure à 450 mvolt, on ajoute des agents stabilisant le potentiel rédox du lait, on inocule le lait avec des bactéries lactiques, on fermente le lait jusqu'à l'obtention d'au moins  $10^6$  cfu/ml et une Aw supérieure à 0,97. Utilisation d'un lait ayant un potentiel rédox à 25 °C inférieur à 450 mvolt et contenant des agents stabilisant ce potentiel rédox du lait, pour la préparation d'un produit laitier fermenté par des bactéries lactiques. Compositions laitières conditionnées dans un matériau imperméable ou semi-perméable à l'oxygène, lesdites compositions ayant un potentiel rédox inférieur à 450 mvolt et une Aw supérieure à 0,97, et au moins  $10^6$  cfu/ml de bactéries lactiques probiotiques.

(57) The invention concerns a method for making a fermented milk composition which consists in: pasteurising the milk at a temperature and for a duration such that its redox potential at 25 °C is brought down to a value less than 450 mvolt, adding agents for stabilising the milk redox, inoculating the milk with lactic bacteria, fermenting the milk until at least  $10^6$  cfu/ml and an Aw higher than 0.97 is obtained. The invention also concerns the use of milk having a redox potential at 25 °C less than 450 mvolt and containing agents stabilising this milk redox potential, for preparing a milk product fermented with lactic bacteria. The invention further concerns milk compositions packaged in a material permeable or semipermeable to oxygen, said compositions having a redox potential less than 450 mvolt and an Aw higher than 0.97, and at least  $10^6$  cfu/ml of probiotic lactic bacteria.



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(71) Déposant (pour tous les Etats désignés sauf US): SOCIETE DES PRODUITS NESTLE S.A. [CH/CHI]; Case postale 353, CH-1800 Vevey (CH).  (72) Inventeur; et (75) Inventeur/Déposant (US seulement): MORETON, Rodney, Stuart [GB/FR]; 16, route de Dauville, Oilly le Vicomte, F-14100 Lisieux (FR).  (74) Mandataire: GROS, Florent; 55, avenue Nestlé, CH-1800 Vevey (CH).																												
<p>(54) Title: FERMENTED MILK PRODUCT</p> <p>(54) Titre: PRODUIT LAITIER FERMENTÉ</p> <p>(57) Abstract</p> <p>The invention concerns a method for making a fermented milk composition which consists in: pasteurising the milk at a temperature and for a duration such that its redox potential at 25 °C is brought down to a value less than 450 mvolt, adding agents for stabilising the milk redox, inoculating the milk with lactic bacteria, fermenting the milk until at least 10<sup>6</sup> cfu/ml and an Aw higher than 0.97 is obtained. The invention also concerns the use of milk having a redox potential at 25 °C less than 450 mvolt and containing agents stabilising this milk redox potential, for preparing a milk product fermented with lactic bacteria. The invention further concerns milk compositions packaged in a material permeable or semipermeable to oxygen, said compositions having a redox potential less than 450 mvolt and an Aw higher than 0.97, and at least 10<sup>6</sup> cfu/ml of probiotic lactic bacteria.</p>																												
<table border="1"> <caption>Data from the bar chart showing the number of cells of CMCN-I-STRAIN (cfu/ml) for various thermal treatments and storage conditions.</caption> <thead> <tr> <th>THERMAL TREATMENT</th> <th>STORING: 1 DAY; PACKAGING: SEMIPERMEABLE</th> <th>STORING: 28 DAYS; PACKAGING: SEMIPERMEABLE</th> <th>STORING: 1 DAY; PACKAGING: IMPERMEABLE</th> <th>STORING: 28 DAYS; PACKAGING: IMPERMEABLE</th> </tr> </thead> <tbody> <tr> <td>63°C/30'</td> <td>2,05E+07</td> <td>5,40E+07</td> <td>2,20E+05</td> <td>7,30E+05</td> </tr> <tr> <td>95°C/30'</td> <td>1,00E+08</td> <td>1,00E+08</td> <td>5,00E+07</td> <td>2,80E+07</td> </tr> <tr> <td>121°C/15'</td> <td>1,20E+08</td> <td>1,20E+08</td> <td>4,50E+06</td> <td>6,70E+07</td> </tr> <tr> <td>121°C/60'</td> <td>1,80E+08</td> <td>1,80E+08</td> <td>3,40E+08</td> <td>1,10E+08</td> </tr> </tbody> </table>				THERMAL TREATMENT	STORING: 1 DAY; PACKAGING: SEMIPERMEABLE	STORING: 28 DAYS; PACKAGING: SEMIPERMEABLE	STORING: 1 DAY; PACKAGING: IMPERMEABLE	STORING: 28 DAYS; PACKAGING: IMPERMEABLE	63°C/30'	2,05E+07	5,40E+07	2,20E+05	7,30E+05	95°C/30'	1,00E+08	1,00E+08	5,00E+07	2,80E+07	121°C/15'	1,20E+08	1,20E+08	4,50E+06	6,70E+07	121°C/60'	1,80E+08	1,80E+08	3,40E+08	1,10E+08
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### **Fermented dairy product**

The subject of the present invention is a new process for the preparation of a fermented milk composition, the said process making it possible to obtain food compositions having a particularly high load of lactic acid bacteria, even after packaging and storage for prolonged periods.

10      **State of the art**

Although lactic acid bacteria are generally known to have beneficial effects on human health, only certain categories of lactic acid bacteria are really capable of adhering to the human intestinal cells, of excluding pathogenic bacteria from human intestinal cells, and/or of acting on the human immune system by allowing it to react more strongly to external attacks. Lactic acid bacteria are termed "probiotic" if they possess at least one of these characteristics.

20      To date, relatively few lactic acid bacteria are truly probiotic bacteria. For example, the strains *Lactobacillus casei* ATCC53103, *Lactobacillus acidophilus* CNCM I-1225, *Bifidobacterium breve* CNCM I-1226, *Bifidobacterium infantis* CNCM I-1227 and *Bifidobacterium longum* CNCM I-1228, have thus been scientifically recognized as being probiotic bacteria since they are capable of adhering to human intestinal cells, of excluding pathogenic bacteria from human intestinal cells, and of acting on the human immune system J. of Dairy Science, 78, 491-497, 1995; Applied Env. Microb., 59, 4121-4128, 1993).

35      The probiotic lactic acid bacteria are often also extremely sensitive to oxygen because of their adaptation to the anaerobic living conditions found in the intestinal tract. Furthermore, these bacteria grow poorly in milk, which poses problems for obtaining a sufficient level of lactic acid bacteria in a fermented dairy product.

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To enhance the growth of slow-growing lactic acid bacteria, EP154614 suggests increasing the load of the starter culture during the inoculation of a milk, and also adding to this milk growth activators such as yeast extracts or whey proteins, for example.

5 Although it is possible to solve, by this means, the problems of growth in milk, the probiotic lactic acid bacteria unfortunately still remain very sensitive to the conditions for the processing and  
10 preservation of a fermented milk. Indeed, most of the plastic packagings used to package dairy products are permeable to oxygen. Furthermore, the subsequent processing of a fermented milk, for example to fromage  
15 blanc or to liquid acidified milks, requires stirring of the milk in the presence of air, which increases the level of oxygen in the final product.

20 The present invention aims to overcome the disadvantages of the prior art by providing a process which promotes the survival of lactic acid bacteria.

#### Summary of the invention

25 To this effect, the present invention relates to a process for the manufacture of a fermented milk composition in which a milk is heat-treated at a temperature and for a period such that its redox potential at 25°C is reduced to a value of less than 450 mvolt, and the milk is inoculated with lactic acid  
30 bacteria.

The present invention also covers all the milk compositions packaged in a material which is impermeable or semipermeable to oxygen, the said compositions comprising at least  $10^6$  cfu/ml of probiotic lactic acid bacteria and a redox potential of less than 450 mvolt (cfu comes from the expression "colony forming unit").

35 Likewise, the invention also relates to the use of a milk having a redox potential at 25°C which is less than 450 mvolt, for the preparation of a dairy product comprising lactic acid bacteria.

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Finally, the invention also relates to the use of a fermented dairy composition derived from the present process in the preparation of a dairy product comprising lactic acid bacteria.

5           Against all expectations, the lactic acid bacteria which are known to be sensitive to the conditions for fermentation and preservation of a milk, for example which are sensitive to the presence of air, in fact become more resistant to these conditions, and in  
10          particular become tolerant to the presence of air, as long as the redox potential of the milk in which they live is less than about 450 mvolt. This resistance results in a better bacterial development during the  
15          fermentation of a milk, and a better survival of the bacteria during the preservation of the fermented milk.

Although this redox potential can be adjusted by various means, it has been found that a prolonged pasteurization of the milk is sufficient to obtain a required redox potential. Indeed, a prolonged heat  
20          treatment makes it possible to break certain milk proteins and thus release reducing groups. Furthermore, this treatment makes it possible to cause the proteins and the milk sugars to react so that the reducing compounds derived from Maillard reactions are formed.  
25

This pasteurization has other advantages. Firstly, a prolonged treatment of the milk at high temperatures promotes degassing of the milk, and therefore a low oxygen content in the milk. Secondly, this treatment makes it possible to convert a portion of  
30          the milk lactose to lactulose which is known to stimulate the growth of certain lactic acid bacteria.

Brief description of the figures

35          - Figure 1 represents, after fermentation, the number of cells of the CNCM I-1225 strain (cfu/ml) having grown in various milks, the said milks having undergone various heat treatments before fermentation, the said fermented milks having been stored for 1 or 28 days at refrigeration temperatures, and the said fermented milks

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having also been packaged in glass or polystyrene packagings.

5       - Figure 2 represents, after fermentation, the number of cells of the CNCM I-1225 strain (cfu/ml) having grown in various milks, the said milks having a redox potential of the order of 500 mvolt or -50 mvolt before fermentation, and the said fermentation having been carried out under aerobic or anaerobic conditions.

10      - Figure 3 represents the number of cells of the CNCM I-1225 strain (cfu/ml) having grown in various milks, as well as the redox potential at 25°C of these fermented milks, as a function of the duration of storage of these fermented milks at refrigeration temperatures.

15      Detailed description of the invention

Within the framework of the present invention, the name "rennet" is given to the coagulating extract obtained from the abomasum of young ruminants slaughtered before weaning. It will be assumed that rennet also covers calf rennet substitutes such as animal pepsins; the coagulating preparations obtained from the plant kingdom extracted from artichoke, thistle, ficin, latex, fig, papain, for example; the coagulating preparations obtained from the microbial kingdom extracted from bacteria of the genus *Bacillus* and *Pseudomonas*, and moulds belonging to the species *Endothia parasitica*, *Mucor pusillus* and *Mucor miehei*, for example.

30      Milk is intended to designate, on the one hand, a milk of animal origin, such as cow's, goat's, sheep's, buffalo's, zebra's, horse's, ass's and camel's milk and the like. This milk may be a milk in the native state, a reconstituted milk, a skimmed milk, or a milk supplemented with compounds necessary for the growth of the bacteria or for the treatment of the milk, such as 35 fats, yeast extract, peptone, ascorbic acid and/or a surfactant, for example. Preferably, these milks have a pH of the order of 6.4-7, in particular pH 6.6-6.8.

The term milk also applies to what is commonly called a vegetable milk, that is to say an extract of

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plant materials, treated or otherwise, such as legumes (soya bean, chickpea, lentil and the like) or oilseeds (rapeseed, soya bean, sesame, cotton and the like), which extract contains proteins in solution or in colloidal suspension, coagulable by chemical action, by acid fermentation and/or by heat. It has been possible to subject these vegetable milks to heat treatments similar to those for animal milks. It has also been possible to subject them to treatments which are specific to them, such as decolorization, deodorization, and treatments for eliminating undesirable tastes. Finally, the word milk also designates mixtures of animal milks and of vegetable milks. Preferably, these milks have a pH of the order of 6.4-7, in particular pH 6.6-6.8.

Against all expectations, it has been found that the growth and the survival of certain lactic acid bacteria are also influenced by the water activity of the milk ( $Aw$ ), that is to say the ratio between the partial vapour pressure of the water at the surface of the powder and the vapour pressure of pure water at the same temperature. The best survival levels may be obtained when the  $Aw$  of the milk at  $20^{\circ}\text{C}$  is greater than 0.97, preferably between 0.988-0.983, for example. As a guide, the  $Aw$  may be determined by measuring the equilibrium relative humidity reached in a closed vessel at constant temperature. For that, a sample of a few g of milk is enclosed in a leaktight container placed in a thermostatted chamber at  $20^{\circ}\text{C}$ . The empty space around this sample reaches, at equilibrium, after 30-60 min, the same  $Aw$  value as the sample. An electronic sensor, mounted in the lid of the container, then measures the humidity in this empty space by means of an electrolytic resistance.

Likewise, it has been found that the addition, to the milk, of at least one bacterial growth promoting agent made it possible to substantially increase the growth and survival of certain lactic acid bacteria. Among these agents, there may be mentioned in particular a sugar such as glucose and sucrose, an amino acid such

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as cysteine and glutathione, a yeast extract, in particular an extract comprising large quantities of purine and pyrimidine bases as well as their phosphate derivatives (adenosine, thymine, guanine, cytosine and uracil) and/or hydrolysates of animal or plant (soya) protein materials, for example. In particular, the milk may comprise about 0.1-1% of yeast extract and/or about 0.25-1% of peptones.

Because it has been realized that the redox potential of a dairy product comprising lactic acid bacteria is capable of being substantially increased during the storage of the dairy product at refrigeration temperatures, it is also preferable to add to the milk compounds which are capable of stabilizing its redox potential. Among these compounds, there may be mentioned all dietary reducing agents such as ascorbic acid, vitamin E and/or their derivatives, which can be used in an amount of 0.01-1% by weight, for example.

The compounds which stabilize the redox potential and the agents which promote bacterial growth may be added to the milk before pasteurization. However, because some of these compounds can be destroyed or even modified following a prolonged heat treatment, it is also possible to envisage adding them to the milk after pasteurization and/or after fermentation, in the form of a sterile solution, for example.

All the devices intended for pasteurizing a milk may be used by persons skilled in the art. It is thus possible to heat treat the milk at at least 90°C for at least 30 min, preferably at 95-130°C for 30-120 min, so as to obtain a redox potential of less than 450 mvolt, in particular of less than 400 mvolt, or even of less than 350 mvolt if it is desired to obtain maximum growth and survival of the lactic acid bacteria, for example.

Next, the pasteurized milk is inoculated with at least one strain of lactic acid bacteria so as to directly obtain in the milk from  $10^3$  to  $10^8$  cfu/ml. It is possible to inoculate the milk with a fresh culture of lactic acid bacteria, with a concentrated and frozen

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culture, or even with a culture dried by lyophilization or by spraying under a stream of hot air, for example.

The strain of lactic acid bacteria may be chosen from the species *Lactococcus lactis* in particular *L. lactis* subsp. *cremoris*, *L. lactis* subsp. *lactic biovar diacetylactis*, and *L. lactis*; *Streptococcus thermophilus*; the acidophilic bacteria comprising *L. acidophilus*, *L. crispatus*, *L. amylovorus*, *L. gallinarum*, *L. gasseri*, *L. johnsonii*; *Lactobacillus fermentum*; *Lactobacillus casei* in particular *L. casei* subsp. *casei*; *Lactobacillus delbrueckii* in particular *L. delbrueckii* subsp. *lactis*; *L. delbrueckii* subsp. *helveticus*; *L. delbrueckii* subsp. *bulgaricus*; the bifidobacteria, in particular *Bifidobacterium infantis*, *Bifidobacterium breve*; *Bifidobacterium longum*; and finally *Leuconostoc mesenteroides* in particular *L. mesenteroides* subsp. *cremoris*, for example (Bergey's Manual of Systematic Bacteriology, Vol. 2, 1986; Fujisawa et al., Int. Syst. Bact., 42, 487-491, 1992).

Preferably, lactic acid bacteria are used which are sensitive to oxygen, in particular all the bifidobacteria, *Lactobacillus acidophilus*, *Lactobacillus johnsonii*, *Lactobacillus gasseri*, *Lactobacillus fermentum*, *Lactobacillus casei*, *Lactobacillus bulgaricus* and *Lactobacillus helveticus*.

The probiotic bacteria are of special interest within the framework of the present invention. These bacteria are in fact capable of adhering to human intestinal cells, of excluding pathogenic bacteria from human intestinal cells, and of acting on the human immune system by allowing it to react more strongly to external attacks (immunomodulation capacity), for example by increasing the phagocytosis capacities of the granulocytes derived from human blood (J. of Dairy Science, 78, 491-497, 1995: immunomodulation capacity of the La-1 strain which has been deposited at the Pasteur Institute under the number CNCM I-1225).

By way of example, it is possible to use the *Lactobacillus acidophilus* CNCM I-1225 strain described in

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EP577904. This strain was recently reclassified among the *Lactobacillus johnsonii*, following the new taxonomy, proposed by Fujisawa et al., which is now authoritative in the field of taxonomy of acidophilic lactobacilli  
5 (Int. J. Syst. Bact., 42, 487-791, 1992).

The milk composition obtained by the process according to the invention may also be traditionally fermented until at least  $10^6$  cfu/ml, in particular  $10^7$ - $10^9$  cfu/ml, are obtained, for example. When these milk  
10 compositions comprise probiotic lactic acid bacteria, in particular the *L. johnsonii* CNCM I-1225 strain, it is preferable to carry out the fermentation in the absence of oxygen, for example under a carbon dioxide atmosphere.

The milk composition obtained by the process according to the invention may also be converted to unripened fromage frais which are commonly called "quarg" or "cottage cheese" in Anglo-Saxon countries and "quark" in Germany, for example. For that, it is possible to ferment the milk inoculated with lactic acid bacteria,  
15 but not necessarily. Rennet, of the order of 0.01 to 0.15% by volume/volume, is generally added to it so as to cause the casein to pass from a colloidal phase to a precipitated phase, this passage being accompanied by the formation of a whey. Next, the whey is separated by  
20 centrifugation or ultrafiltration.  
25

The invention also covers all milk compositions packaged in a material which is impermeable or semipermeable to oxygen, the said compositions comprising at least  $10^6$  cfu/ml of probiotic lactic acid bacteria and a redox potential of less than 450 mvolt, preferably of less than 400-350 mvolt if compositions are desired in which the viability of the lactic acid bacteria is stabilized at an acceptable level.

Preferably, the milk compositions according to the invention are packaged in a material which allows the passage of less than  $0.01 \text{ cm}^3$  of air per day and per  $\text{cm}^2$  under an external pressure of 0.21 bar, for example a material which is impermeable to air such as glass or ethyl vinyl alcohol (EVOH), or a material which is

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semipermeable to air such as polystyrene (PS), polypropylene (PP), polyethylene terephthalate (PET), ethyl vinyl alcohol (EVOH), high-density polyethylene (HDPE), or a mixture of these materials, for example.

5           Because the lactic acid bacteria which exist in a milk treated according to the present process become particularly resistant to stress situations, the milk composition obtained by the present process can also be used to prepare other fermented dairy products, in  
10           particular as a starter for a fermentation of a milk on a large scale, for example.

15           The present invention is described in greater detail below with the aid of the additional description which follows, which refers to examples of preparation of  
20           fermented dairy products, as well as to the description of a test for measuring the redox potential. The percentages and parts are given by weight unless otherwise stated. It goes without saying, however, that these examples are given by way of illustration of the subject of the invention and do not constitute in any manner a limitation thereto.

#### Measurement of the redox potential

25           The measurement of the redox potential is carried out in accordance with the publication by Buhler H. et al. (Ingold A.G., Germany). For that, a pH/mvolt-meter combined with a redox electrode (Ingold No. 105053288) is used. The pH/mvolt-meter is calibrated using a standard redox buffer. The milk samples at pH  
30           6.4-7 are incubated beforehand on a bath at 25°C. The measurement of the redox potential is carried out after 3 min of stability, and the redox potential is calculated by adding 244.4 mvolt to the redox value displayed.

#### Example 1

Several milk samples consisting of 10% of a skimmed milk powder, 1% of yeast extracts and 0.5% of glucose are prepared. In order to obtain redox potentials of less than 450 mvolt, these milks are heat-treated,

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respectively, for 30 min at 63°C on a hot water bath, for  
30 min at 95°C on a hot water bath, for 15 min at 121°C  
in an autoclave, or for 60 min at 121°C in an autoclave.  
These milks are inoculated with the probiotic strain  
5      *Lactobacillus johnsonii* CNCM I-1225 which was deposited  
at the Pasteur Institute, 25 rue du docteur Roux, Paris,  
30 June 1992. These milks are fermented without stirring  
until a pH of the order of 4.6 is obtained, they are each  
10     packaged in two impermeable (glass) or semipermeable  
packagings, each packaged milk is stored for 1 day or 28  
days at refrigeration temperatures, and then after  
storage, the number of lactic acid bacteria which survive  
is determined.

15     The results presented in Figure 1 clearly show  
that the prolonged heat treatment of the milk  
substantially enhances the survival of the lactic acid  
bacteria, even in the presence of oxygen. Moreover, it is  
also observed that the redox potentials of the heat-  
treated milks are inversely correlated with time and with  
20     the temperature level applied to the milk. In other  
words, the more extensive the heat treatment of the milk,  
the lower the redox potential of the milk. In this  
regard, it should thus be noted that the lower the redox  
potential of the milk, the more manifest the resistance  
25     of the lactic acid bacteria.

#### Example 2

30     The redox potentials at 25°C of two artificial  
MRS media are adjusted respectively to about 500 mvolt  
and -50 mvolt, by adding thereto an appropriate quantity  
of potassium ferricyanide or of DTT. These two media are  
inoculated with an inoculum of the *Lactobacillus*  
*johnsonii* CNCM I-1225 strain, and they are fermented  
under aerobic conditions. For the aerobic conditions,  
35     sterile air bubbles are introduced into the fermentation  
media. Finally, the number of bacterial colonies which  
have grown in these milks is counted.

The results presented in Figure 2 show that the  
milks having a redox potential at 25°C of the order of

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-50 mvolt give the best growth scores, whether in the absence or in the presence of air. Consequently, when the redox potential of a milk is reduced, the growth of certain lactic acid bacteria is thereby promoted.

5

Example 3

Two starters are prepared from the *Lactobacillus johnsonii* CNCM I-1225 strain and from the *Streptococcus thermophilus* CNCM I-1421 strain which was deposited at the Pasteur Institute, 25 rue du docteur Roux, Paris, 18 May 1994, in a milk consisting of 10% of a skimmed milk powder, 1% of yeast extract and 0.5% of glucose, the said milk having previously been heat-treated at 95°C for 30 min.

15

A skimmed milk conventionally pasteurized at 115°C for 20 min is inoculated with 5% of the *Lactobacillus johnsonii* CNCM I-1225 strain starter and with 0.5% of the *Streptococcus thermophilus* CNCM I-1421 strain starter. When the pH of the fermented milks reaches pH 4.5, 0.1% weight/volume of vitamin C is added, the milks are packaged in pots which are semipermeable to air and they are stored at refrigeration temperatures for 1, 14 or 28 days, after which the redox potential of the fermented milks is measured and the number of *Lactobacillus johnsonii* CNCM I-1225 bacteria which have survived in these fermented milks is counted.

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For comparison, a milk is fermented under the same conditions, the only difference being that vitamin C is not added.

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The results presented in Figure 3 show that if the redox potential of a fermented milk is reduced to and stabilized at less than 450 mvolt, a survival rate of the lactic acid bacteria of at least 50% is obtained after 28 days of storage; whereas if the redox potential of the fermented milk is greater than 450 mvolt, less than 1% survival is obtained after 28 days of storage.

Example 4

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Two milks consisting of 10% of a skimmed milk powder and various concentrations of yeast extracts are prepared, these media are heat-treated at 115°C for 15 min, they are inoculated with 5% of a fresh culture of the *Lactobacillus johnsonii* CNCM I-1225 strain, they are incubated at 40°C for 1 to 28 days, and the number of lactic acid bacteria which have survived these storage conditions is determined.

For comparison, milks fermented and stored for 1 to 28 days are analysed, the said milks comprising no yeast extracts.

The results show that the use of 0.1 to 1% of yeast extracts in the culture medium promotes the survival of the lactic acid bacteria during prolonged storage of this medium. The best results are obtained for milks having of the order of 1% of yeast extracts.

Example 5

Several milks containing 10% of a skimmed milk powder and various concentrations of additives are prepared, these media are heat-treated at 115°C for 15 min, they are inoculated with 5% of a fresh culture of the *Lactobacillus johnsonii* CNCM I-1225 strain, they are incubated at 40°C until a pH of 4.5 is obtained, they are cooled to 4°C for 28 days in pots which are permeable to air or semipermeable to air, and the number of lactic acid bacteria which have survived these storage conditions is determined. The experimental conditions are given in Table 1 below. It should however be noted that vitamin C is added after fermentation and before storage, in the form of a sterile solution.

The results presented in Table 1 below show that the peptone extracts, the yeast extracts and/or the vitamin C make it possible to enhance the survival of the probiotic lactic acid bacteria after 28 days of storage at refrigeration temperatures, even in the presence of air.

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Table 1

Media	Packaging	% survival after 28 days
10% of a skimmed milk powder, 1% of yeast extract, 0.5% of glucose	permeable semipermeable	0.22 14.03
10% of a skimmed milk powder, 1% of yeast extract, 0.5% of glucose, and 0.01% of vitamin C	permeable semipermeable	2.47 21.18
10% of a skimmed milk powder, 1% of yeast extract, 0.25% of peptone, and 0.01% of vitamin C	permeable semipermeable	0.34 10.55
10% of a skimmed milk powder, 1% of yeast extract, 0.25% of peptone, and 0.1% of vitamin C	permeable semipermeable	1.72 21.11
10% of a skimmed milk powder, 1% of yeast extract, 0.25% of peptone, and 0.5% of vitamin C	permeable semipermeable	28.09 32.85
10% of a skimmed milk powder, 1% of yeast extract, 0.25% of peptone, and 1% of vitamin C	permeable semipermeable	31.81 31.81

Example 6

5 Several milks are prepared which consist of 10% of a skimmed milk powder, 1% of yeast extracts and various concentrations of sucrose so as to adjust the water activity of the milk from 0.978 to 0.989. These media are heat-treated at 115°C for 15 min, they are inoculated  
 10 with 5% of a fresh culture of the *Lactobacillus johnsonii* CNCM I-1225 strain, they are incubated at 40°C until a pH of 4.5 is obtained, they are cooled to 4°C, they are stored at 4°C for 1 to 28 days in pots which are semipermeable to air, and the number of lactic acid  
 15 bacteria which have survived these storage conditions is determined.

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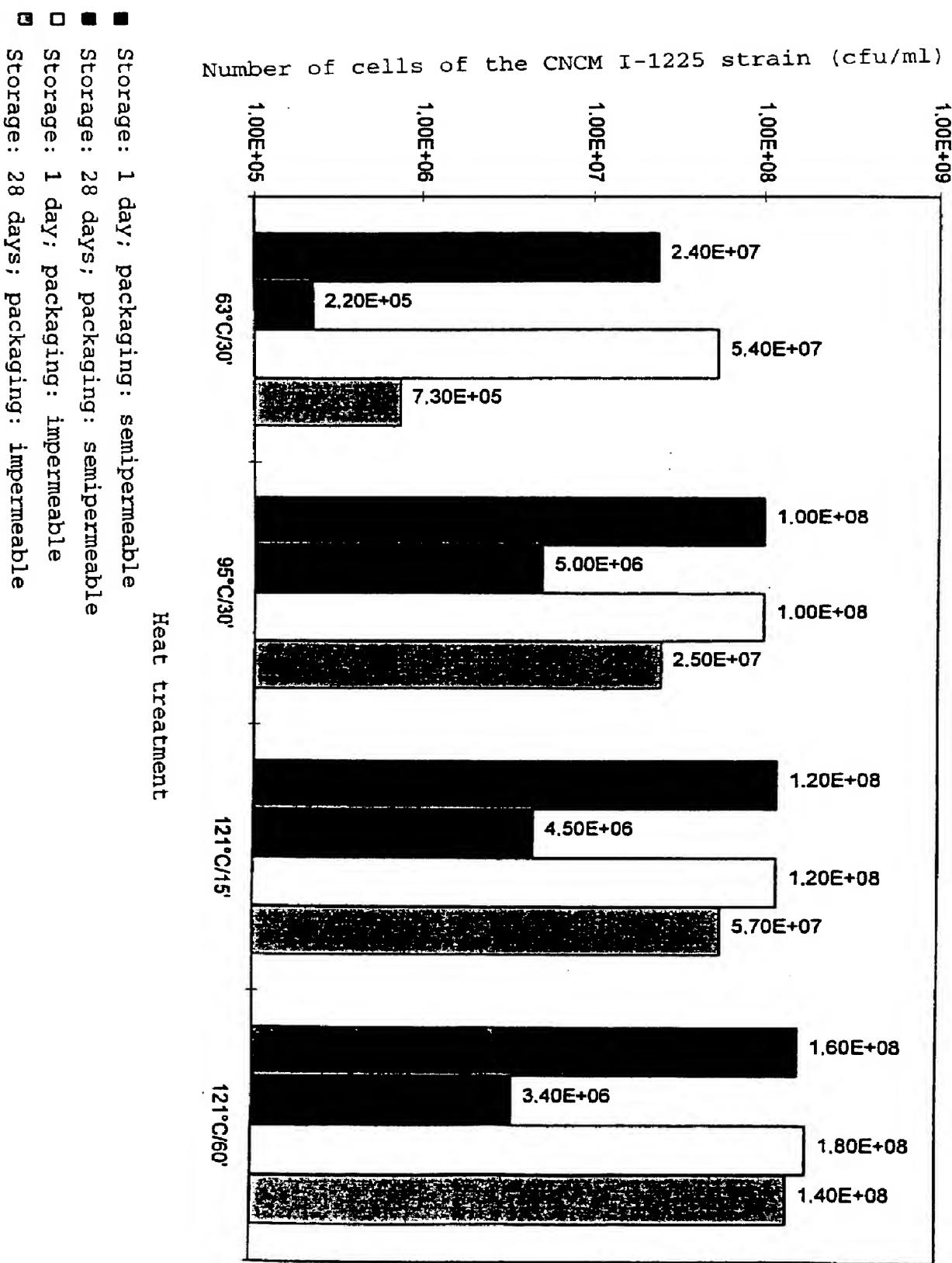
The results show that the lactic acid bacteria survive storage better for a prolonged period at refrigeration temperatures when the Aw of the culture medium before fermentation is of the order of 0.985.

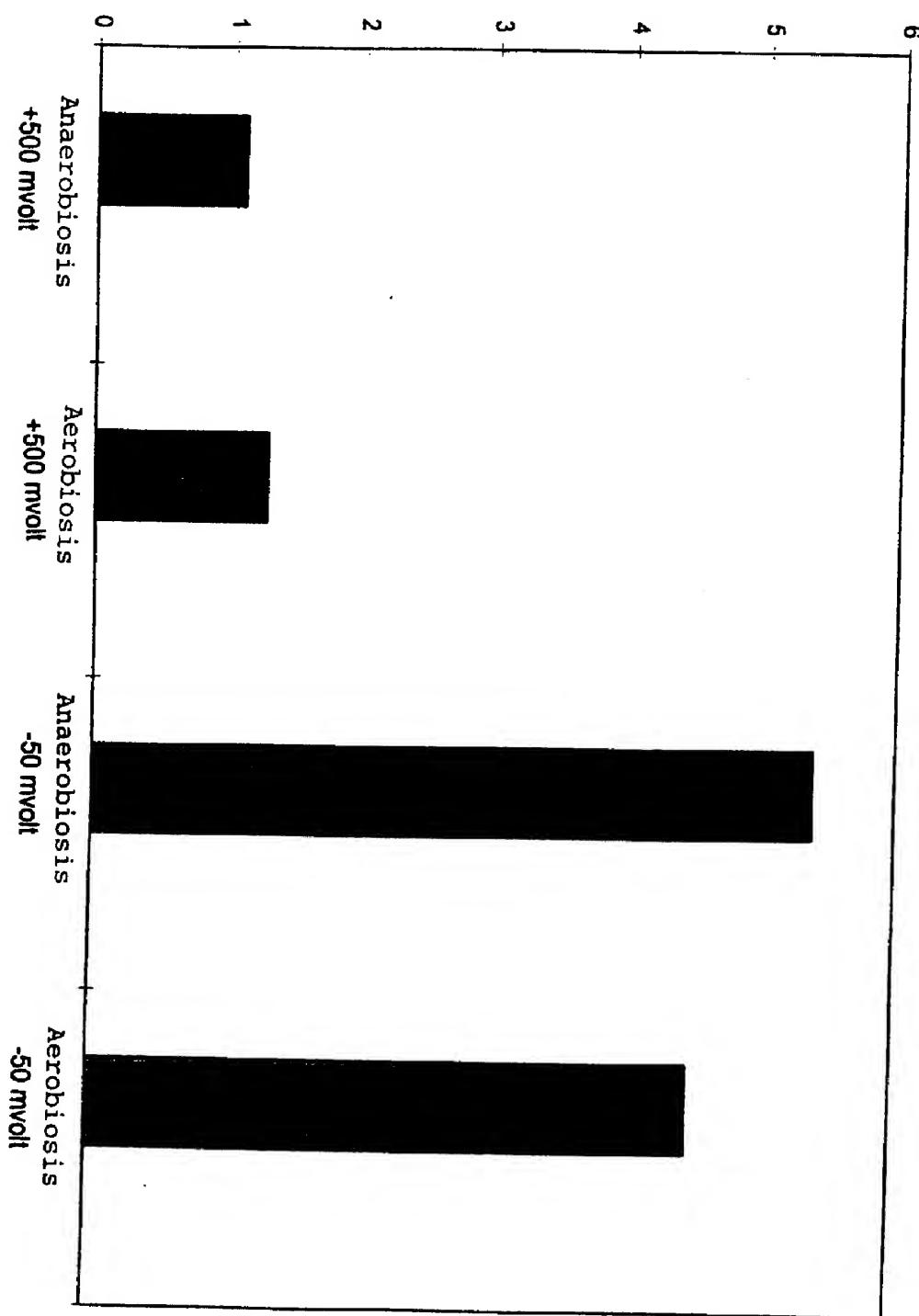
- 15 -

Claims

1. Process for the manufacture of a fermented milk composition in which a milk is pasteurized at a temperature and for a period such that its redox potential at 25°C is reduced to a value of less than 450 mvolt, agents which stabilize the redox potential of the milk are added, the milk is inoculated with lactic acid bacteria, the milk is fermented until at least  $10^6$  cfu/ml and an Aw greater than 0.97 are obtained.
- 5 2. Process according to Claim 1, in which the milk comprises, in addition, bacterial growth promoting agents.
- 10 3. Process according to either of Claims 1 and 2, in which the milk is fermented by lactic acid bacteria capable of adhering to human intestinal cells, of excluding pathogenic bacteria from human intestinal cells, and of acting on the human immune system by allowing it to react more strongly to external attacks.
- 15 4. Use of a milk having a redox potential at 25°C of less than 450 mvolt and containing agents which stabilize this redox potential of the milk, for the preparation of a fermented dairy product by lactic acid bacteria.
- 20 5. Use according to Claim 4, for the preparation of a dairy product packaged in a material which is impermeable or semipermeable to oxygen.
- 25 6. Milk compositions packaged in a material which is impermeable or semipermeable to oxygen, the said compositions having a redox potential of less than 450 mvolt and an Aw greater than 0.97, and at least  $10^6$  cfu/ml of probiotic lactic acid bacteria.

Figure 1



Number of cells of the CNCM I-1225 strain  $\times 10^8$  (cfu/ml)

Growth conditions

Figure 2

Figure 3

